# The Mis-identification of Anatoxin-a Using Mass Spectrometry in the Forensic Investigation of Acute Neurotoxic Poisoning

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## Introduction

Anatoxin-a (AN) is a potent neurotoxin, produced by a number of cyanobacterial species. Forensic investigations of suspected AN poisonings are frequently hampered by difficulties in detecting this toxin in biological matrices due to its rapid decay. Further impediments are the lack of availability of AN analogues and their degradation products. Possible confusion can also occur in identifying AN as the causative agent in both human and animal fatalities due to the presence of the amino acid Phenylalanine (Phe).

## Hypothesis

Nano-electrospray hybrid quadrupole time-of-flight (nano ESI QqTOF) MS can accurately differentiate AN and Phe.

## Method

In July 2002 a suspected human intoxication in the USA, that relied on liquid chromatography - single quadrupole MS (LC-MS), confused Phe and AN, since both have similar masses. We previously developed a quadrupole ion-trap (QIT) MS for the determination of AN in cyanobacteria and drinking water. Liquid chromatography-multiple tandem mass spectrometry (LC-MS$^n$) was employed to study the fragmentation pathway of Phe, in positive mode, to identify characteristic product ions and fragmentation processes.

## Results

Reversed-phase LC, using a $C_{18}$ Luna column gave similar retention times and on certain C18 columns can co-elute. The molecular related species [M+H]$^+$ *m/z* 166 was used as the precursor ion for LC-MS$^n$ experiments. MS$^2$-MS$^4$ spectra displayed major characteristic product ions for Phe. Fragmentation of other adduct ions [M+Na]$^+$ and [M+NH$_3$] were examined in order to identify distinctive product ions. A comparison of the QIT MS$^n$ data for AN and Phe can prevent misidentification. Nano-electrospray hybrid quadrupole time-of-flight (nano ESI QqTOF) MS was then used to confirm formulae assignments of the product ions using high mass accuracy data and to identify ions in the lower mass range.

## Conclusion

Nano-electrospray hybrid quadrupole time-of-flight (nano ESI QqTOF) MS can confirm the formulae assignments of the product ions using high mass accuracy data and to identify ions in the lower mass range.